JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2002, p. 3889–3893 Vol. 40, No. 11
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.11.3889–3893.2002

GUEST COMMENTARY

Clinical Microbiology in the Year 2025

W. Michael Dunne, Jr.,[1]* J. Keith Pinckard,[1] and Lora V. Hooper[2]

Departments of Pathology and Immunology[1] and Molecular Biology and Pharmacology,[2] Washington University School of Medicine, St. Louis, Missouri 63110

This article offers a mini-preview of what's to come in the field of clinical microbiology, and it's the first such undertaking that any of the authors has ever attempted without tongue firmly planted in cheek. Obviously, the scenario portrayed herein is an exercise in pure fantasy, based loosely on the evolutionary pace of clinical microbiology witnessed over the past 25 years. Unlike readers of *Sports Illustrated*'s yearly predictions of champions and losers, however, the reader will have to wait longer than a single season to prove us right or wrong (23 years to be exact). Had the task been the opposite—i.e., to reminisce about the state of clinical microbiology a quarter century ago—we would have discussed the introduction of the Analytab Products bacterial identification system or the first-generation AutoMicrobic system (AMS, Vitek Systems, Inc.) originally designed for use in the U.S. space program. We could generate a smirk by recollecting that the role of the clinical microbiologist in the mid-1970s was to identify all microbial life forms recovered from clinical specimens and then to provide susceptibility test results for each by disk diffusion testing. It was the clinician who would then sort through the myriad of results and decide which organism(s) deserved a therapeutic response. We could prompt a grimace or two by recalling that blood cultures were monitored visually for evidence of bacterial growth once or twice per day and that all anaerobes were identified to species level no matter how much time or how many biochemical reactions were required to do the trick. Interestingly, the two aspects of clinical microbiology that haven't changed much since the mid-1970s are the identification of fungal diseases and the identification of parasitic diseases.

As a discipline and profession, clinical microbiology has "come a long way baby" (Virginia Slims, circa 1970), but we have just begun the molecular diagnostics learning curve, and it's hard to predict just how far we can take this new tool from a technical, practical, and economic standpoint. Because of the recent explosion of technology, many clinical microbiologists have openly speculated that ours is a dying profession—one that will ultimately be consumed by the growing molecular diagnostic beast. With that in mind, here's a mythical take on the future of the clinical microbiology laboratory. One additional note: this might represent the only publication in the history of the *Journal of Clinical Microbiology* that contains no references. Why is this? The references that would have been cited haven't been written yet!

* Corresponding author. Mailing address: Departments of Pathology and Immunology, and Molecular Microbiology, Washington University School of Medicine, Medical Director of Microbiology, Barnes-Jewish Hospital, St. Louis, MO 63110. Phone: (314) 362-1547. Fax: (314) 362-1461. E-mail dunne@labmed.wustl.edu.

THE YEAR 2025

B. Jeffrey Lane, M.D., is a primary care physician and part of a busy group practice in suburban St. Louis. He joined the practice 3 years earlier in June of 2022 after completing a combined family practice residency-MBA program at an East Coast medical college. Dr. Lane typically sees about 20 patients per day, works 4 days a week and one-half day on Saturday every other week. In addition, he handles between 10 and 15 patient interactions per day online via his wireless pocket patient manager (the Curbstone VII), leaving little time for his financial and managerial responsibilities within the practice. Despite a burgeoning patient volume, Dr. Lane's workload has been aided considerably this past year by the purchase of several key instruments for use by the group practice. Most prominent of these are the TransVue whole-body-imaging array and the CyberPath on-site clinical laboratory system. The latter was purchased with the addition of the Shylock bloodless hematology-thrombostasis analyzer and five MyCrobe real-time, hand-held infectious disease diagnostic units (affectionately referred to by the staff as "bug boy advance").

On this particular Monday morning (the day after Super Bowl LIX in which the Detroit Lions defeated the Portland Cardinals), Dr. Lane faces a full slate of patients. His first patient is a 16-year-old young man with fever, headache, nausea, and severe pharyngitis with exudate. After a careful examination and history, Dr. Lane removes a sterile collection kit for the MyCrobe system and plugs the tip into the battery-operated handle. The tip resembles an old-fashioned swab like the ones that were once used to collect microbiology specimens, but the distal end is made of a porous material with microfibers on the surface and a hollow shaft that connects the tip to a bulb containing sterile processing buffer. Dr. Lane places the tip of the device against the tonsillar pillars and presses the green button on the grip. A small amount of sterile buffer is expressed through the shaft and to the surface of the porous, bristled tip that produces gentle sonication while the tip rotates. After a brief period of contact, he presses the red button that activates a small vacuum and aspirates the sample back into the porous tip. Dr. Lane removes a MyCrobe UR (upper respiratory) cassette from the foil pouch, places the tip of the collection device into a port on the top of the cassette, and presses a snap-lock trigger that severs the tip into the port

and seals the cassette. Dr. Lane places the cassette into the clip on the back of his hand-held MyCrobe module. The results will be available in approximately 15 min, so the patient is instructed to remain in the examination room pending the need for therapy. The results of the assay will appear simultaneously on the screen of the hand-held device and on the patient's electronic chart, which is linked by a wireless interface. Billing and diagnostic codes are automatically linked to the result and transmitted to the office's financial management system.

Once the MyCrobe UR cassette has been placed into the analyzer, the instrument initiates a cascade of sample processing activities. The processing buffer used to collect the sample has already begun to lyse both eukaryotic and prokaryotic cells. The buffer contains a mixture of detergents and enzymes that promote the release but prevent the degradation of nucleic acids. Once in the sample port, the buffer is expressed from the collection device, and the resultant lysate is diverted into two chambers.

The first chamber is a nucleic acid processing station where released DNA and/or RNA is captured by a mixture of target-specific MolecuLures bound to microspheres, each representing multiple target sequences (including resistance and virulence genes) of 168 potential bacterial, viral, fungal, and parasitic agents of upper respiratory tract infection. Captured DNA and/or RNA is subjected to rapid (20 cycles/min) electropulse isothermal amplification. Amplified products are hybridized in the same chamber to 1 or more of 8,000 target probes located at specific positions on a polymer-coated grid. Hybridization produces a change in the electrical potential of the polymer at the location of a specific probe that is proportional to the amount of DNA and/or RNA present in the original specimen. The signals thus generated are decoded and translated into a probability list of potential etiologies coinciding with a large database, and, if a matching signal(s) is detected, a virtual susceptibility report based on the detection of resistance genes is developed.

The second chamber has been designed to extract and process proteins, glycoproteins, and carbohydrates released in the specimen lysate. The creation of this aspect of the UR cassette was prompted by several highly publicized attacks in the early 2000s in which bioterrorist groups released environmental and biological toxins via aerosols that caused acute upper respiratory tract illness but were not detected by nucleic acid-based assays. Protein, glycoprotein, and exopolysaccharide antigens are initially digested into peptides, glycopeptides, and carbohydrate subunits. Similar to the process used in the nucleic acid detection system, antigens are allowed to react with a matrix of ligands derived from HLA class II proteins and anchored to a second polymer reporter grid. A signal pattern is produced that is proportional to the affinity of each ligand for the antigens present in the specimen. This, in turn, is compared to an extensive database of signal pattern possibilities to construct a list of potential infectious etiologies, toxins, or resistance factors such as β-lactamases, modified penicillin binding proteins, capsular antigens, pili, flagella, or hemagglutinins. MyCrobe also correlates results obtained from both chambers of the system to determine whether gene sequences encoding resistance factors are actually transcribed into functional enzymes, toxins, or capsules. After a result is generated, the cassette can be stored at room temperature indefinitely for additional evaluation, such as complete genome sequencing. In addition to the UR cassette, the U.S. Food and Drug Administration has cleared a number of specimen-specific cassettes for use with the MyCrobe system. These include a cassette for agents of gastrointestinal disorders, including food-borne illnesses; a cassette for use with blood, cerebrospinal fluid, and peritoneal fluid, etc.; a cassette for the diagnosis of urinary tract infections (UTIs) and sexually transmitted infections; and a cassette for use with sputum, bronchoalveolar lavage fluid, and tracheal secretions. Each cassette has been formulated to provide a representation of a vast number of microorganisms most likely to be associated with specimen-specific infectious processes with a fair degree of overlap. For example, sequences generated by amplification of *Mycobacterium tuberculosis* targets are represented on the sterile fluid, lower respiratory tract, urinary tract, and stool cassettes. Similarly, *Streptococcus pneumoniae* targets are represented on the matrix constructed for both the lower respiratory and sterile fluid cassettes. Amplification of human immunodeficiency virus-associated targets (qualitative, quantitative, and resistance sequences) are available on the sterile fluid and UTI-sexually transmitted disease cassettes, and written consent is no longer required following the passage of the new patient's rights bill in 2021. While the CyberPath Corporation had initially considered providing geographically specific modifications to cassettes sold in various locations throughout the world to reflect regional differences in infectious diseases, they concluded that a universal approach would be more practical because of the abundance of worldwide travel. Any of the aforementioned cassettes can be used with tissue biopsy specimens, provided the sample has been processed first with one of the many commercial tissue digestion-extraction kits.

In the case of this patient, 15 DNA and RNA sequences specific for *Streptococcus pyogenes* were detected in sufficient quantity to suggest a diagnosis of streptococcal pharyngitis. The organism was determined to harbor macrolide-lincosamide-streptogramin B resistance secondary to the detection of the *ermTR* gene sequence and is also resistant to β-lactam antibiotics because of the expression of a common molecular class A β-lactamase (the first such strain was reported in Spain in 2015). Hyaluronic acid capsular antigen, group A-specific carbohydrate antigen, and M type 3 antigen were detected, but none of the pyrogenic exotoxins were identified by ligand binding despite signal amplification of a *speA* gene sequence in the nucleic acid processing chamber. This pattern of group A streptococcus had been the one most commonly encountered by Dr. Lane over the past year.

In addition to detecting *S. pyogenes*, the MyCrobe UR detects low-level signals for coronavirus RNA of the B814 strain type and the coronavirus S protein, suggesting recent past or acute infection. No Epstein-Barr virus-associated DNA or RNA sequences or antigens are detected. The patient is prescribed a β-lactam–β-lactamase inhibitor combination and is instructed to e-mail his progress throughout the week.

Dr. Lane's second patient of the day is a 53-year-old woman whom he had seen 5 days earlier for a UTI. At that time, MyCrobe analysis of a urine sample placed in the UTI card had detected *Escherichia coli* genomic targets at a predicted level of 86,000 copies/ml of urine (considered significant). The system also detected markers of a drug-resistant dihydropteroate syn-

thase, a class B metallo β-lactamase, a class C inducible β-lactamase, and a TEM-347 extended-spectrum β-lactamase that predicted overall resistance to sulfa antibiotics, carbapenems, and first- through sixth-generation cephalosporins. However, no determinants of quinolone resistance were identified with respect to currently recognized mutations of DNA gyrase or topoisomerase IV sequences or the presence of quinolone efflux pumps. Amplification products indicative of P and type 1 fimbria genes were also detected, and both corresponding antigens were recognized on the ligand side of the UTI card, but gene sequences encoding Shiga toxins or hemolysins were not amplified. Dr. Lane had prescribed a 3-day course of omnifloxacin—a newly released bromo-fluoroquinolone that achieves extremely high levels in the urine. The patient insisted that she had religiously taken all doses of the antibiotic and yet had felt no symptomatic relief. Dr. Lane collected another urine sample from the woman and repeated the MyCrobe UTI analysis. Fifteen minutes later the display provided the same results that had been obtained earlier in the week. Perplexed by the lack of response to omnifloxacin by a seemingly susceptible organism, Dr. Lane instructed his assistant to send an aliquot of the urine specimen as well as the UTI card to the regional medical center laboratory for further analysis.

The clinical laboratory of the regional medical center serves as the reference laboratory for primary care clinics and community hospitals in eastern Missouri and west-central Illinois as well as providing extensive diagnostic testing for patients hospitalized at the center's adult and pediatric hospitals. The microbiology section is staffed by two associate directors (one with an M.D. and one with a Ph.D.) and employs five medical technologists and five laboratory assistants. One of the directors has been trained as a traditional clinical microbiologist with specialty training in microbial terrorism, while the other has a background in molecular microbiology and genetics. Two of the medical technologists have attained a master's degree in molecular technology, while the other three have received specialist certification in medical and molecular microbiology. The laboratory is equipped with two MyCrobe Magna units as well as all equipment and materials necessary for the cultivation, differentiation, and preservation of microorganisms. The MyCrobe Magna performs all of the screening tests available with the hand-held devices but, in addition, provides for total genomic sequencing of purified bacterial DNA, viral DNA and RNA, and partial sequencing of fungal and parasite genomes. In addition, the instrument is capable of performing gene expression analysis with concomitant sequencing of all upregulated mRNA produced when a microorganism is grown in the presence of an antimicrobial agent. All sequences derived from the Magna system are compared to a large international database that stores information on common, novel, or rare resistance and virulence factors. Epidemiological data derived from molecular typing are used to update regional, national, and international databases concerning the prevalence and geographic location of strains. Data are also transmitted to the Centers for Disease Control and Prevention for publication in the *Morbidity and Mortality Biweekly Report*, which is published online. It was a first-generation MyCrobe Magna system that was used to identify the first glycopeptide-resistant *Clostridium difficile* strain in Brazil 5 years earlier.

The urine specimen forwarded from Dr. Lane's patient was cultured on agar-based media, similar to the way routine cultures were performed at the turn of the century. The media included both selective and nonselective agar plates, a plate with chromogenic substrates, and a plate with inhibitory levels of omnifloxacin for gene expression analysis as suggested from the data downloaded from Dr. Lane's MyCrobe report. In addition, total DNA and RNA were extracted from the specimen and lyophilized for future analysis.

As predicted by the UTI card, a single lactose-positive, gram-negative rod was recovered from cultures of the urine sample at a cell density exceeding 75,000 CFU/ml. The organism was identified as *E. coli* by phenotypic characteristics and whole-genome analysis. The latter also indicated that this strain was one of the six most common genotypes isolated from clinical samples in the region. No other previously recognized resistance mutations or factors were identified using the genome sequencing function of the MyCrobe Magna. Expression analysis of cells grown in the presence of omnifloxacin at 8 μg/ml provided the first clue for the resistance mechanism. One transcript coding for the expression of an outer membrane protein porin (OmpF) was markedly upregulated. Sequence analysis of this transcript disclosed mutations leading to three predicted amino acid substitutions in this porin. Two of these, P226Y and T269G, were adjacent to the opening of the OmpF channel. The third, E117N, occurred in the constricted portion of the hourglass-shaped channel and altered the overall charge in that environment. To confirm this finding, reconstitution of the wild-type *ompF* gene into the isolate completely restored susceptibility to omnifloxacin, and cloning of the mutant *ompF* into a fully omnifloxacin-susceptible strain of *E. coli* produced transformants for which the MICs of omnifloxacin were >16 μg/ml. The data derived from this study were entered into the MyCrobe Magna database for future reference and for possible inclusion on regularly scheduled updates of the UTI card.

The regional microbiology laboratory at the medical center receives approximately 350 specimens per week for confirmation of virtual susceptibility and identification results using this type of high-level molecular analysis with the MyCrobe Magna system combined with traditional culture and other research tools and protocols. Laboratory personnel maintain complete competence in culture techniques and stock all materials required for the recovery of aerobic and anaerobic bacteria, fungi, and viruses from clinical specimens for this very purpose. Sequence analysis of rRNA genes has also been successfully applied toward the identification of common yeasts, systemic fungi, dermatophytes, hyphomycetes, and zygomycetes. However, sequences from a large number of saprophytic or opportunistic fungi have not yet been determined. As a result, fungal cultures still remain the mainstay of diagnostic mycology.

Much of the microbiology laboratory workload at the reference facility is devoted toward the analysis of "MyCrobe-ambiguous" results. These specimens are usually obtained from patients with suspected infection who have been analyzed at primary care clinics, and usually no nucleic acid or antigen targets are detected in these specimens by the point-of-care MyCrobe system. The reference microbiology laboratory is also called upon to reconcile incompatible MyCrobe results, e.g., when virulence targets are identified but do not correspond with the appropriate organism identification. An exam-

ple of the latter might be the detection of exotoxin A without amplification of *Pseudomonas aeruginosa* targets or the identification of *S. pneumoniae* targets that suggest that the isolate is fully susceptible to quinolones or β-lactam antibiotics (strains such as these haven't been recognized since 2015). Once again, the reference laboratory combines both old and new technology by relying on culture as well as extended genomic-proteomic analysis using the MyCrobe Magna system. On more than one occasion, the laboratory has identified virulence factors long associated with one species as being unexpectedly produced by another. In one well-publicized case, a staphylococcal enterotoxin gene was purposefully introduced into a *Lactobacillus* species by a radical animal rights group. A member of the group who had gained employment in a meat packaging plant then contaminated multiple lots of hamburger with the bioengineered organism. The contaminant went undetected by molecular screens designed to identify the presence of food-borne bacterial pathogens but not toxins. Several weeks later, reports of "enterotoxin-positive, *Staphylococcus aureus*-negative" MyCrobe results from stool and food samples led to extended culture evaluation by the reference laboratory. The enterotoxin-producing *Lactobacillus* was eventually discovered and the source of the hamburger was identified.

There have been several interesting developments in the field of laboratory medicine and clinical microbiology following the release of the CyberPath system and three other competing point-of-care diagnostic modules. First, the impact of these technologies significantly reversed the trend of centralized laboratory services popularized in the late 1990s and early 2000s. The rapid turnaround time for specimen analysis and the diagnostic accuracy of the expansive test menus provided by these systems proved to be extremely popular among physicians and patients alike. Test results became available before the patient left the examination room, thereby improving the continuum of care and hastening the administration of appropriate therapeutic agents. The increasing number of units placed in primary care clinics and community hospitals throughout the country began to slowly erode test volumes and profits of commercial reference laboratories and regional medical center laboratories alike. Indeed, various modules of the CyberPath system and its competitors were finding their way into surgical suites, intensive care units, and emergency departments. This, in turn, prompted a major shift in the business strategy of large commercial or central laboratories from high-volume, moderate-complexity testing facilities to highly specialized analytical laboratories concentrating almost exclusively on complex genomics- and proteomics-based analyses. Ultimately, those commercial and central laboratories that successfully adapted to the new market required dramatic changes in the educational level of their personnel. The skills necessary to design, perform, and interpret specialized testing now performed at these sites required staffing expertise on par with that of the biotechnical industry, having a much higher proportion of individuals with advanced degrees in molecular biology, genetics, and bioengineering.

Another major change that took place as testing was moved from centralized laboratories to the physician's office was that physicians performing diagnostic testing on site had to become certified in office laboratory medicine. Although the use of advanced and sophisticated molecular testing instrumentation is now considered routine, it is still classified as point-of-care testing and, as such, requires a Clinical Laboratory Improvement Act license for its use. And because of the sheer volume of diagnostic information generated by these systems, Clinical Laboratory Improvement Act 2017 has mandated more rigorous standards for compliance with quality assurance and quality control (QA/QC) issues. For example, continuing medical education in QA/QC protocols is required of all users on an annual basis as part of the certification process. QC reagents are built into the MyCrobe service contract and play a role in an exchange program in which the manufacturer ships 10 QC-proficiency samples to users on a biweekly basis. The results are automatically transmitted to a central repository to ensure instrument performance and precision. QC-proficiency samples contain a mixture of recombinant DNA, RNA, protein, and carbohydrate designed to survey all of the possible targets detectable by the system over a 3-month period. In the case of a discrepant result, instructions for a detailed investigation are sent by e-mail to the client and the offending unit is removed from use pending additional studies. The QA/QC program also plays a role in a reciprocity agreement whereby clients submit random patient samples to the manufacturer for comparative analysis. The user will be locked out of the system unless QC-proficiency samples are run according to scheduled input. In the event of a shipping error or other complications, the manufacturer is capable of sending a temporary override signal to reactivate the instruments. Criteria for acceptable performance of MyCrobe users has been established by the College of International Pathologists and mandates 90% agreement with expected target detection for each shipment of QC-proficiency samples.

Interestingly, despite the advance in diagnostic technology, it has been necessary to maintain proficiency at the reference laboratory in "old-school" microbiology skills such as culture, identification, and susceptibility testing of bacteria, fungi, parasites, and viruses. At the current level of development, systems like the MyCrobe cannot resolve all discrepancies between genotype and phenotype, and the organism identification database is far from complete. In fact, the rapid advance of molecular diagnostics in the field of clinical microbiology has served to preserve the past as well as ensuring the future of the profession—at least for the next 25 years.

EPILOGUE

Our take on the future provides a happy ending for the profession of clinical microbiology. Perhaps these predictions will come true such that the advanced diagnostic technology will continually rely on the basic principles and practice of culture and identification, as we know it today. Perhaps this is too shortsighted and technological development will advance at a far greater pace than predicted. Ours is not the only profession that is wrestling with the prospect of extinction. Surgical pathologists will insist that a skilled individual and a microscope can never be replaced by a microarray for the diagnosis of all disease states. Certainly, the diagnosis of a large number of infectious diseases (and diseases in general) will be made using molecular technology in the year 2025, but we contend that the proportion of microorganisms amenable

to this approach will never reach 100%. Further, the emergence of unique infectious diseases or novel mechanisms of resistance will always cause us to haul the old tools out of the closet until these agents have been identified, characterized, and inserted into version 17.1 of the updated database. Hey, if we're wrong, call us in 2025.

The views expressed in this Commentary do not necessarily reflect the views of the journal or of ASM.